Isolation and Identification of Environmental Mycobacteria in the Mycobacterium bovis BCG Trial Area of South India

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The isolation profiles of environmental mycobacteria present in soil, water, and dust samples, and sputum samples of persons with symptoms of chest infection in the South Indian Mycobacterium bovis BCG (bacillus Calmette-Guérin) trial area were compared. Isolates belonging to the Mycobacterium avium-intracellulare-scrophulaceum complex were predominant in water, dust, and sputum samples and Mycobacterium fortuitum-complex organisms were predominant in soil samples irrespective of the season of the year.

The highly variable results with respect to the protective efficacy of Mycobacterium bovis BCG (bacillus Calmette-Guérin) (range, 0 to 80%) (9) in major BCG trials have raised important questions regarding the immune mechanisms in tuberculin, the nature of protection offered by BCG, and the rule played by environmental mycobacteria (29). In the south-eastern United States, sensitization to nontuberculous mycobacteria (NTM) (4) has been found to be associated with the occurrence of these NTM in the environment (1, 23). Experimental work with animals has shown that infections with some NTM can offer a level of protection similar to that offered by BCG against tuberculosis (5, 17, 18, 20, 24) and that, depending on the nature of the NTM and the timing, this exposure can also enhance, mask, or interfere with the effect of subsequent BCG vaccination (25, 26). Thus, regional differences in environmental mycobacterial flora along with several other mechanisms could be responsible for the widely varying results of BCG trials (10) or could influence the course of subsequent infection with virulent tubercle bacilli (19).

In the large-scale BCG trial conducted in South India during 1968 to 1980, BCG did not offer any protection against the bacillary form of pulmonary tuberculosis (28). The study population in this that was was characterised by a high prevalence of tuberculous infection and disease and also by a very high prevalence of nonspecific sensitivity (16, 28, 30). Further, 20% of the NTM obtained from sputum samples of subjects in this area (in 1 year) belonged to the Mycobacterium avium-intracellulare-scrophulaceum (MAIS) complex (21). One of the several hypotheses put forward to explain the results of this trial suggests that prior exposure to NTM, which is present in the environment of this area, could have played a role in inodulating the immune response to subsequent BCG vaccination (29). Systematic investigations of the isolation and identification of mycobacteria from the environment of the area and comparison of such a mycobacterial profile with that for BCG (bacillus Calmette-Guérin) trial area were conducted. Isolates belonging to the Mycobacterium avium-intracellulare-scrophulaceum complex were predominant in water, dust, and sputum samples and Mycobacterium fortuitum-complex organisms were predominant in soil samples irrespective of the season of the year.
were aged 10 years and above and had either symptoms of chest infection or histories of previous treatment.

Statistical analysis. Chi-square and Fisher’s exact tests were used to test for any association between the profiles, both within each type of sample at the two time-points and among the different types of samples at each time-point. Kappa and McNamer’s tests were used for paired comparisons of samples from the same sites at the two time points (11).

Table 1 presents the culture results obtained from soil, water, and house-dust samples collected in January and June from the same sites in the study area. The highest number of mycobacterial isolates was obtained from soil samples; the lowest number was obtained from dust. The proportion of positive samples from soil, water, and dust was lower in June than in January. This reduction was statistically significant (P = 0.046) in the case of soil and highly significant (P < 0.001) in the case of water.

Among the positive samples, the majority yielded one or two species, with maximums of five and four species, respectively, yielded by positive soil and water samples. The proportion of samples yielding only one species was significantly increased in June as compared with January in the case of water samples (P = 0.01) but not in the case of soil samples (P > 0.2 and P = 0.2, respectively).

Table 2 presents the percentages of samples yielding different mycobacterial species. In a few cases, the isolates could not be identified up to species level with the procedures used; these have been grouped as “others” among the slow growers and rapid growers.

Both soil and water samples yielded a greater proportion of pathogenic mycobacteria than non-pathogenic mycobacteria in both January and June. This was not seen in the case of sputum samples. Non-pathogenic mycobacteria and MAIS-complex organisms were isolated from a significantly greater (P < 0.0001 and P < 0.01, respectively) percentage of water samples in January than in June, even though the differences in the proportions of samples yielding pathogenic and non-pathogenic mycobacteria were not significant (P > 0.2 and P = 0.1, respectively) for soil or water.

The significant reductions in June, as compared with January, both in the proportions of soil, water, and dust samples positive for mycobacteria and in the numbers of MAIS-complex isolates and nonpathogens in water samples, suggest that climate could play an important role in the distribution of environmental mycobacteria. The high ambient temperature (ranging from 35 to 40°C) in the study area in June could be one of the reasons for the reduction in the number of positive samples among samples collected during this period.

The results indicate that in this area, the mycobacterial isolation profile for water most resembles the profile for sputum samples in certain aspects. For instance, from both water and sputum, organisms belonging to the MAIS complex were the most frequently isolated in both January and June. There was no significant difference (P = 0.3) in the percentages of water and sputum samples yielding MAIS-complex organisms in June. Though there was a significant difference (P = 0.02) in the percentages of water and sputum samples yielding MAIS-complex organisms in January, it must be recalled that there was a significant reduction in June, as compared with January, in the proportion of water samples (P < 0.01) but not sputum samples (P > 0.2) yielding MAIS-complex organisms. The frequent occurrence of MAIS-complex organisms in sputum samples in the present study is in conformity with the findings of an earlier study in this area (21) in which MAIS-complex organisms were also the most frequently isolated (22.6% of all NM).

The comparison of isolation profiles from water and sputum samples also shows that isolates belonging to the Mycobacterium fortuitum complex are few in both cases. The proportions of water and sputum samples yielding M. fortuitum-complex organisms were not significantly different in either January (P = 0.3) or June (P = 0.2).

The isolation profile for soil is markedly different from the isolation profiles for water and sputum. Isolates belonging to the M. fortuitum complex were predominant in soil. M. fortuitum-complex isolates were yielded by significantly higher proportions of soil samples (P < 0.0001) than of water or sputum samples in both January and June. On the other hand, the proportion of samples yielding MAIS-complex isolates was significantly lower for soil samples than for water and sputum samples in both January and June (P < 0.0001 and P = 0.02, respectively, for water and P < 0.01 and P < 0.0001, respectively, for sputum). Further, isolates belonging to Mycobacterium asiaticum, Mycobacterium gordonae, and Mycobacterium triviale were obtained from water and sputum samples alone.

It has been earlier proposed that inhalation of aerosols containing M. avium, Mycobacterium kansasii, and Mycobacterium xenopi generated in water systems, showers, and sinks may cause infection or colonization of the respiratory tract (2, 3). After experiments to simulate natural aerosolization of M. intracellulare and M. scrofulaceum, Parker et al. (22) reported that M. intracellulare was significantly more concentrated in water droplets than M. scrofulaceum and that the water-to-air pathway could be one of the means of human infection by NTM. The similarity in the isolation profiles of mycobacteria from water and sputum samples in the present study also suggests this possibility.

The isolation profile for dust is interesting, again because of certain similarities with the profile for sputum samples. In an earlier study, M. fortuitum, M. gordonae, and Mycobacterium nonchromogenicum were found to be the most frequently isolated species from dust, followed by M. intracellulare and M. scrofulaceum (27). According to the authors, dusts were the source of mycobacteria occurring in sputa as casual isolates.
and the pathogenic mycobacteria in dusts were more likely to survive in the human respiratory tract in the present study, MAIS-complex organisms were the predominant isolates from dust as well as from sputum and water. Isolates belonging to the \textit{M. fortuitum} complex were less frequently isolated from dust. A few other species were also obtained from dust; they include \textit{Mycobacterium diernhoferi}, \textit{Mycobacterium flavescens}, and \textit{Mycobacterium xenopi}.

Thus, this study indicates that in the South Indian BCG trial area, organisms belonging to the MAIS complex are the predominant nontuberculous mycobacterial species in water and dust samples from the environment and in sputum samples from objects residing in the same area, while organisms belonging to the \textit{M. fortuitum} complex are predominant in soil. It would be of interest to further investigate the isolates belonging to these two complexes.

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